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MASH1 is Highly Specific for Neuroendocrine Carcinoma: An Immunohistochemical Evaluation on Various Neoplastic Tissues

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Introduction

Achaete-scute complex homolog-1 (ASCL1), known as mASH1 in rodents and hASH1 in humans, is a basic helix-loop-helix transcription factor that is critical for neuroendocrine cell differentiation.[1,2] Neuroendocrine carcinomas can arise in different sites such as lung, the gastrointestinal tract, prostate and skin.[2] High grade, poorly differentiated neuroendocrine carcinomas are classified as neuroendocrine carcinomas (NECs) and are distinguished from low grade neuroendocrine tumors (NETs).[2,3] Classic neuroendocrine markers such as Chromogranin A and CD56 cannot distinguish NECs from NETs.[2] Recent studies have shown that MASH1 stains hASH1 in human tissues and can distinguish NECs from NETs in various sites.[3-5] MASH1 has also been shown to distinguish small cell lung carcinoma (SCLC) and large cell neuroendocrine carcinoma (LCNEC) from non-small cell lung cancer (NSCLC).[3-5] MASH1 has also been used to differentiate SCLC from Merkel cell carcinoma.[6] While not a tissue-specific marker, MASH1 may assist in distinguishing NECs from NETs in poorly differentiated cases.[2] To date, no comprehensive immunohistochemistry (IHC) studies with MASH1 on various normal and non-NEC/NET neoplastic tissues have been reported. In lung cancers, only limited studies on MASH1 specificity in lung adenocarcinoma and lung squamous cell carcinoma have been reported. The aim of this study is to evaluate the specificity and sensitivity of MASH1 in various neoplastic tissues, with emphasis on non-NEC/NET cancers, SCLC and NSCLC.

Materials and Methods

Formalin-fixed paraffin-embedded tissue microarrays (TMAs) were used consisting of normal tissues (n = 33), various neoplastic tissues (n = 650) and lung cancers (n = 250). Tissues were cut at 4µm and placed on positively charged slides. Slides were deparaffinized and hydrated down to water, placed in a modified citrate buffer solution and heated in a pressure cooker at 110 °C for 15 minutes. MASH1 [24B72D11.1] (Biocare Medical, Concord, CA) titer was optimized at 1:200, followed by a polymer detection system and visualized with 3,3'-Diaminobenzidine (DAB) chromogen. MASH1, Chromogranin A and CD56 were also compared in a side by side study in 23 cases of SCLC. A double stain cocktail of mouse monoclonal MASH1 (DAB) and a rabbit monoclonal Chromogranin A (Fast Red) was also developed for IHC and evaluated in SCLC.

Results

Results are summarized in Tables 1 and 2. In normal tissues, MASH1 (nuclear staining) was expressed in C-cells in thyroid and in epithelial cells in thymus. All other normal tissues were negative including astrocytes, normal adrenal, pancreas and argentaffin cells found in the gastrointestinal tract.

In NSCLC, MASH1 stained 1% (1/91) of squamous cell carcinomas, 1% (1/87) of adenocarcinomas and 0% (0/30) of large cell carcinomas. MASH1 stained 82% (67/82) of SCLC and 67% (2/3) of LCNEC (Table 1; Figures 1A, 1B). In the side by side study of 23 cases of SCLC, MASH1, Chromogranin A and CD56 stained 83% (19/23), 78% (18/23) and 91% (21/23), respectively. The antibody double stain cocktail of MASH1 (nuclear, DAB) and Chromogranin A (cytoplasmic, Fast Red) gave identical results as the individual stains (Figure 1C). In various other neoplastic tissues, MASH1 was expressed in thyroid medullary carcinomas (Figure 2A), in astrocytomas/glioblastomas (Figure 2B) and in thymic carcinomas (Figure 2C) but not in thyroid papillary and follicular carcinomas (Table 2).(Figure 2B) and in thymic carcinomas (Figure 2C) but not in thyroid papillary and follicular carcinomas (Table 2).

Discussion

MASH1 was shown to be highly specific in lung NEC vs. NSCLC (Table 1). However, MASH1 stained 1% (1/87) and 1% (1/94) of lung adenocarcinoma and lung squamous cell carcinoma cases, respectively. Both cases were confirmed by immunohistochemistry and thus a rare subtype of lung adenocarcinoma with neuroendocrine differentiation was observed. These findings were also observed by Ionescu *et al.* However, the authors concluded that NSCLC with neuroendocrine differentiation should not be a subclass distinct from the other NSCLC, because the disease specific and overall survival was not influenced by neuroendocrine differentiation.[7] Our data supports that MASH1 is not expressed in most non-NET/NEC cancers. MASH1 was positive in only 4% (26/650) of neoplastic cases, and MASH1 expression was only found in cases of astrocytoma, glioblastoma, thymic carcinoma and thyroid medullary carcinoma.

Neuroendocrine cancers can show overlapping morphological features independent of their site of origin. These overlapping features can make the identification of the primary location problematic, especially when they are cancers of unknown origin.[2] In normal tissues, MASH1 was observed in normal thyroid C-cells and in epithelial cells in thymus.

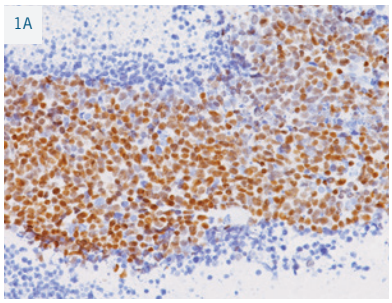
Discussion (Continued)

Medullary thyroid cancer is one of the neuroendocrine cancers derived from the thyroid C-cells and hASH1 has been shown to be highly expressed in neuroendocrine tumors such as medullary thyroid cancer.[8] In our study, MASH1 was expressed in 48% (15/31) of thyroid medullary carcinoma and in 42% (5/12) of thymic carcinoma (Table 2). All type A, AB and B1-B3 thymomas were negative for MASH1 (Table 2). Type C thymic carcinoma with neuroendocrine differentiation has been previously reported.[9]

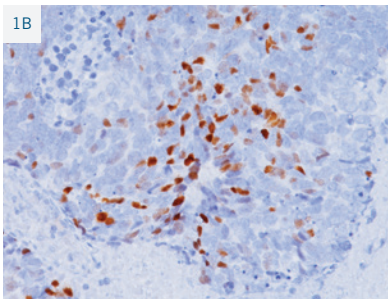
CD56, Chromogranin A and synaptophysin have been the most reliable immunohistochemical markers to detect neuroendocrine differentiation in tumors of unknown primary and in neuroendocrine lung tumors.[10-15] However, as previously discussed, this panel of neuroendocrine markers cannot distinguish NECs from NETs. In a report by La Rosa *et al*, achaete-scute homolog 1 (MASH1) was not detected in any gastroenteropancreatic neuroendocrine tumors, was expressed in only a minority of lung carcinoids and was identified in 70% of extrapulmonary neuroendocrine carcinomas.[2] In a report by Shida *et al*, the authors revealed that MASH1 was highly (sensitivity of 71%) and specifically (specificity of 95%) expressed in poorly differentiated neuroendocrine carcinoma and did not stain gastroenteropancreatic carcinoids.[16]

In various types of sarcomas, MASH1 was negative in all 84 cases (Table 2). This may be significant as other neuroendocrine markers have been known to be expressed in soft tissue tumors such as rhabdomyosarcoma, carcinosarcoma, leiomyosarcoma and synovial sarcoma.[17-20] Even though, in normal brain, MASH1 was negative, it was noted that MASH1 was expressed in astrocytoma and glioblastoma (Table 2). This data is supported by Somasundaram *et al* as their study revealed that MASH1 was overexpressed in progressive astrocytoma as evidenced by increased levels of MASH1 in higher grades.[21]

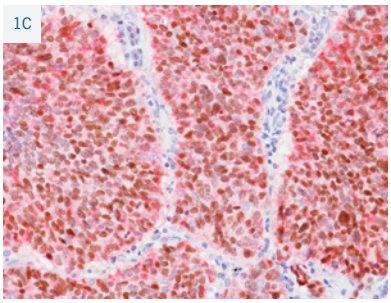
Figure 1



Small Cell Lung Cancer stained with MASH1

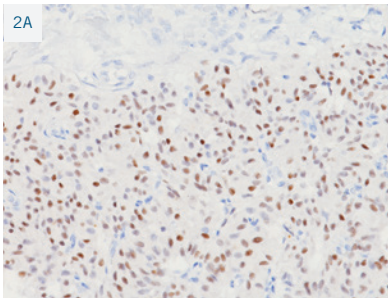


Neuroendocrine Large Cell Carcinoma stained with MASH1

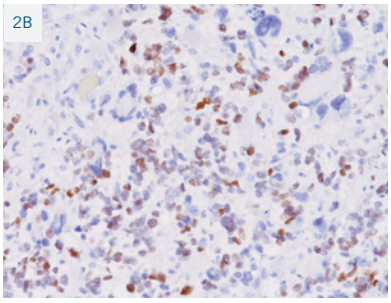


MASH1 and Chromogranin A Double Stain in SCLC

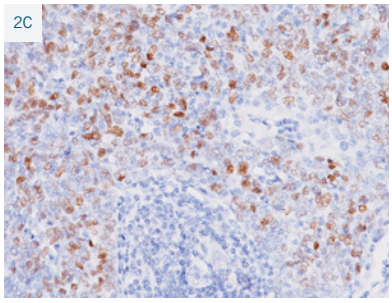
Figure 2



Medullary Thyroid Cancer stained with MASH1



Glioblastoma Grade IV stained with MASH1



Thymic Carcinoma stained with MASH1

Table 1: MASH1 Expression in Lung Cancer (n = 250)

Lung Cancers	MASH1	% (+)
NSCLC		
Adenocarcinoma	1/87	1%
Squamous cell	1/91	1%
Classic large cell	0/30	0%
SCLC	67/82	82%
LCNEC	2/3	67%
Typical carcinoid	0/4	0%
Atypical carcinoid	5/12	42%

Table 2: MASH1 Expression in Various Types of Neoplastic Tissues (n = 650)

Tissue Type	(+)	(-)	% (+)
Adrenal cortical adenocarcinoma	0	10	0%
Bladder (urothelial carcinoma)	0	8	0%
Brain cancer (n = 40)			
Astrocytoma	5	28	15%
Glioblastoma	1	6	14%
Breast cancer (infiltrating ductal)	0	17	0%
Cholangiocarcinoma	0	9	0%
Colon adenocarcinoma	0	30	0%
Embryonal tumor (multiple)	0	6	0%
Gastrointestinal carcinoids (various)	0	4	0%
Kidney cancer (renal cell)	0	22	0%
Hepatocellular carcinoma	0	40	0%
Lymphoma	0	17	0%
Melanoma	0	22	0%
Ovarian cancer (serous)	0	12	0%
Pancreatic cancer (n = 35)			
Islet cell carcinoma	0	4	0%
Pancreatic ductal carcinoma	0	21	0%
Pancreatic adenocarcinoma	0	10	0%
Pheochromocytoma	0	2	0%
Prostate adenocarcinoma	0	38	0%
Sarcoma (n = 84)			
Bone (Giant cell tumor)	0	11	0%
Osteosarcoma	0	5	0%
Lyposarcoma	0	15	0%
Fibrosarcoma	0	20	0%
Dermatofibroma protuberans	0	3	0%
Fibrous histiosarcoma	0	4	0%
Rhabdomyosarcoma	0	10	0%
Leiomyosarcoma	0	9	0%
Epithelioid sarcoma	0	2	0%
Synovial sarcoma	0	3	0%
Carcinosarcoma	0	1	0%
Angiosarcoma	0	1	0%
Seminoma	0	70	0%
Thymic tumors (n = 63)			
Thymoma (A, AB, B1, B2, B3)	0	51	0%
Thymic carcinoma	5	7	42%
Thyroid Cancer (n = 41)			
Medullary carcinoma	15	16	48%
Thyroid papillary carcinoma	0	9	0%
Follicular carcinoma	0	1	0%
Uterine Carcinoma (n = 80)			
Squamous cell carcinoma	0	40	0%
Adenocarcinoma	0	30	0%
Adenosquamous cell carcinoma	0	10	0%

Conclusion

Although not organ specific, MASH1 is highly specific for neuroendocrine carcinomas (NECs) of the lung vs. NSCLC and was also shown to be highly discriminating in various types of non-NEC/NET cancers.

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